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Author/Editor: Tilkens et al.

Journal/Book Title: Molec. Biol of the Cell

Article Title: Serum-free mediums

Volume (Issue): 4 (Suppl.)

Pages: 335A

Year of Publication: 1993 Supp. NO 3/1

Publisher:

Remarks:

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Molecular Biology of the Cell

Supplement to Molecular Biology of the Cell Volume 4 Oct 1993

Abstracts

The American Society for Cell Biology
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EPITHELIAL CELLS MODULATE THE VIRULENCE OF *SALMONELLA TYPHI* ((SK Kops, MG Kashgarian, and AB West)), Yale University School of Medicine, New Haven, CT 06510

Salmonella typhi Ty2 traverses Transwell filters bearing confluent monolayers of polarized enterocyte-like CACO-2/$C2_{BBe}$ cells within 15 minutes of application, causing increased permeability as evaluated by fluxes of ^{3}H-mannitol and falling transmonolayer electrical resistance. Intracellularly sequestered *S. typhi* were cultured from $C2_{BBe}$ monolayers, after washing with gentamicin to kill extracellular bacteria. In these experiments most of the bacteria remained extracellular. This was confirmed by ultrastructural studies, immunolabeling *S. typhi* with an antibody to the H (fimbrial) antigen, which showed numerous extracellular bacteria associated with the brush border. Small numbers of bacteria were present within cells in membrane bound vacuoles. In addition, in contrast to controls, many $C2_{BBe}$ cells exposed to *S. typhi* had prominent vacuoles that contained amorphous immunoreactive material probably derived from fimbriae. Quantitative determinations of bacteria in upper and lower chambers demonstrated that 5% of freshly cultured *S. typhi* inoculated into the upper well passed through the monolayer. In contrast, <0.2% of *S. typhi* that were recovered from the lower chamber transmigrated when applied to fresh monolayers. Thus, the interaction of *S. typhi* with $C2_{BBe}$ epithelial cells involves structural and metabolic changes in the bacteria which influence virulence of these pathogens. Moreover, antibiotic-free medium used to culture $C2_{BBe}$ cells in flasks for three days was found to contain a heat-labile factor that inhibited growth of *S. typhi in vitro*. This factor may possibly be a means by which enterocytes act on the bacteria to modulate pathogenicity, and may constitute an innate host defense mechanism.

1943

SERUM-FREE MEDIUM FOR THE GROWTH AND RECOMBINANT PROTEIN PRODUCTION OF ANCHORAGE-DEPENDENT CHINESE HAMSTER OVARY CELLS. ((M.L. Tilkins, P.J. Battista and S.F. Gorfien)) GIBCO BRL/Life Technologies, Inc., Cell Culture R&D, 2086 Grand Island Blvd., Grand Island, NY 14072.

Chinese hamster ovary (CHO) cells are widely used for the expression of foreign genes owing to stable gene expression, and the ability to produce recombinant proteins which are structurally and functionally analogous to the naturally occurring protein. GIBCO BRL currently offers a variety of serum-free media (SFM) formulations designed to support the growth and recombinant protein expression of CHO cells in suspension culture. Modifications have been made to one of these formulations, CHO-S-SFM II, to yield a serum-free prototype specifically designed for anchorage-dependent CHO cells. Adherent CHO SFM is a low-protein, low-endotoxin formulation which contains no bovine-derived components. This prototype formulation has been demonstrated to support growth and recombinant protein production using a variety of anchorage-dependent cell culture systems including: tissue culture flasks, roller bottles, microcarriers and artificial capillary bioreactors. When compared to serum-supplemented cultures, higher cell densities and recombinant protein levels have been achieved in Adherent CHO SFM. Additionally, Adherent CHO SFM has demonstrated its utility in transfection protocols, yielding stable CHO transfectants. The use of serum-free medium eliminates many problems associated with serum, such as lot-to-lot variability, presence of unknown agents, and fluctuations in price and availability. Furthermore, serum-free culture simplifies downstream processing and recovery of recombinant proteins. The features of Adherent CHO SFM make it appealing for the serum-free culture of anchorage-dependent CHO cells.

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APICAL AND BASOLATERAL ACTIVE TRANPSORT SYSTEMS MEDIATING PEPTIDE FLUX ACROSS CACO-2 CELLS. (P.S. Burton, R.A. Conradi, A.R. Hilgers, N.F.H. Ho and C.L. Barsuhn) Drug Delivery Systems Research, Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001

We reported recently the existence of a saturable, apically polarized transport system in Caco-2 cells for peptides which served to hinder apical to basolateral flux, enhance basolateral to apical flux and show substrate specificity (BBRC 190 760 (1993)). This system was further inhibited by verapamil, suggesting some homology with p-glycoprotein, the principal mediator of drug resistance in multi-drug resistant cancer cells. More recently, a polarized uptake system for these same peptides has been identified in the basolateral membrane of Caco-2 cells. Both systems are energy dependent, inhibited by verapamil, and seem to move substrate against a concentration gradient. Upon saturation and/or inhibition with verapamil of the active transport mechanisms, the peptide fluxes in apical to basolateral direction and the basolateral to apical direction converge and become controlled by the passive mechanism which is dependent upon the number of polar functional groups in the peptide which require desolvation before the molecule can transfer from the membrane interfacial region into the apolar membrane interior. The results suggest that, to the extent that the Caco-2 cell serves as a reasonable model for the human intestinal mucosa, these systems would work in tandem to hinder peptide absorption from the lumen while promoting excretion from the systemic circulation.

1945

ENHANCED FATTY-ACID SYNTHASE (FAS) ACTIVITY IN HYPERTROPHIC ALVEOLAR TYPE II CELLS FROM SILICA-TREATED RATS: STUDIES ON FUNCTION AND MESSENGER RNA LEVELS. ((J. Rami, W. Stenzel, C. Puel-M'Rini, J.P. Besombes and S.A. Rooney)). INSERM, CJF.9107, Toulouse 31054, France and Dept. of Pediatrics, Yale University, New Haven, CT 06510.

Hypertrophic type II cells (HTC) from silica treated rats have elevated phospholipid levels and increased activities of FAS and choline-phosphate cytidylyltransferase (CYT), the rate limiting enzyme in phosphatidylcholine biosynthesis. We previously reported that increased CYT activity in fetal lung is mediated by enhanced FAS gene expression with increased synthesis of fatty acids. We have now investigated whether the increase in FAS activity in HTC is accompanied by increased FAS mRNA and if inhibition of fatty acid synthesis blocks the increase in CYT activity. Type II cells were isolated 1-14 days after intratracheal injection of rats with silica and fractionated into normal and HTC by centrifugal elutriation. HTC FAS activity was increased 1 day after silica injection and had reached maximal level by ~3 days while the increase in CYT activity did not occur until day 3 and was not maximal until day 7. The increase in FAS activity was not accompanied by an increase in mRNA. FAS mRNA in HTC 1-14 days after silica injection was 50-75% the level in control type II cells. At the same time γ-actin mRNA and total RNA levels were the same in both control and HTC groups. In vivo administration of hydroxycitrate and inclusion of agaric acid in the culture medium decreased [^{3}H]glucose incorporation into fatty acids by ~60%. The inhibitors did not diminish the stimulatory effect of silica on FAS activity in HTC 3 days after its administration but completely abolished the increase in CYT activity. We conclude that increased CYT activity in HTC is mediated by the increase in FAS but that the increased FAS activity is not due to enhanced gene expression. (Supported by HL-46488).

1946

CULTURE AND CHARACTERIZATION OF NORMAL EPITHELIUM FROM HUMAN BLADDER BIOPSIES. ((A.L. Trifillis, X. Cui, and J.W. Warren)) Depts of Pathology and Medicine, School of Medicine, University of Maryland, Baltimore, MD 21201. (Spon. by J. Strum.)

In vitro culture systems of specific human cell types have become valuable tools in the study of cellular metabolism and pathogenetic mechanisms of toxicity. We report here the culture and characterization of epithelial cells from normal appearing areas of bladder obtained from 8 patients diagnosed with either bladder or prostatic carcinoma. Biopsies (2-4 mm^3) were minced into 1/2 mm^3 pieces, anchored to dishes with glass coverslips, and incubated in Eagle's medium supplemented with 10% fetal calf serum. Within 5 days cells grew out from the explants. Monolayers reached confluence after 6 weeks. Cells of the monolayer were keratin-positive and exhibited typical epithelial cell ultrastructure, including intermediate filaments and numerous desmosomes. Lateral interdigitations, well-developed Golgi and cytoplasmic vesicles bounded by a trilaminar plasma membrane were also noted. Three epithelial cell types comprise the bladder mucosa of all mammalian species studied: basal, intermediary, and superficial. We believe these cells are intermediary cells based on their ultrastructure which was retained after up to seven passages. Cultures of bladder epithelium grown from small amounts of mucosa available from cystoscopic biopsies will be useful in future studies of cellular toxicity, malignant transformation, and susceptibility to microbes.

1947

PURIFICATION & CHARACTERIZATION OF A UTERINE RETINOL-BINDING PROTEIN IN THE BITCH. ((WC Buhi, IM Alvarez, VM Shille, M-J Thatcher, JP Harney and M Cotton)) Departments of OB/GYN, Large Animal Clinical Sciences and Animal Science, University of Florida, Gainesville, FL 32610.

A complex array of proteins are produced by the cyclic and pregnant bitch endometrium during early diestrus. One major protein, canine protein (cP) 6 (23,000 M_r), appears to be up-regulated during days 3-10 of diestrus. Steroid regimens in ovariectomized bitches have shown that estrogen-priming is required for progesterone induction. The objectives of this study were to purify cP6, determine the N-terminal and internal CNBr-generated amino acid sequences and compare to previously identified proteins, RNA, and DNA databases to identify this protein. Protein cP6 was found in endometrial conditioned explant culture medium as well as uterine flushed material during early diestrus (days 3-10). Using a combination of ion-exchange (DEAE-Sepharose) and gel filtration (Sephadex G-100) chromatography, cP6 was purified to homogeneity. Antiserum to human retinol-binding protein (hRPB) will immunoprecipitate protein cP6. Using both chromatographically and immunologically purified cP6, twenty-two and twenty amino acid sequences were determined for the N-terminal and internal fragments of cP6, respectively. The N-terminal sequence suggested that cP6 was an RPB, 81% identity with rabbit RBP, while the internal sequence suggested a similarity to β-lactoglobulin (βLG) (43% identity), both members of the lipocalin family. Amino acid analysis suggested major differences between cP6 and RBP and βLG. Further study indicated that cP6 could bind ^{3}H-retinol. These studies suggest that bitch endometrium produces a RBP-like molecule that may transport retinol to the early embryo/conceptus during rapid growth and development.